Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification
Novobiocin Antimicrobial Supplement

Intended Use
Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification is used with Novobiocin Antimicrobial Supplement in rapidly detecting motile *Salmonella* in feces and food products.

Summary and Explanation
MSRV Medium, Semisolid Modification is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* in feces and food products. The original work on MSRV medium showed that a semi-solid medium in Petri dishes could be used as a rapid and sensitive means of isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.1,2 The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

The medium is recommended by the European Chocolate Manufacturer’s Association. A collaborative study performed with support of the American Cocoa Research Institute and the Canadian Chocolate Manufacturer’s Association resulted in first action adoption of the MSRV method by AOAC International.3

MSRV Medium, Semisolid Modification may be used as a plating medium for isolating *Salmonella* spp. (other than *Salmonella* Typhi and *Salmonella* Paratyphi A) from stool specimens with high sensitivity and specificity.4,5

Principles of the Procedure
Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification contains peptones as carbon and nitrogen sources for general growth requirements. Magnesium chloride raises the osmotic pressure in the medium. Novobiocin (Novobiocin Antimicrobial Supplement) and malachite green inhibit organisms other than *Salmonella*. The low pH of the medium, combined with the novobiocin, malachite green and magnesium chloride, helps to select for highly resistant *Salmonella* spp. Agar is the solidifying agent.

Formulæ

**Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification**

<table>
<thead>
<tr>
<th>Component</th>
<th>Approximate Formula* Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>4.59 g</td>
</tr>
<tr>
<td>Casein Hydrolysate (acid)</td>
<td>4.59 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>7.34 g</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>1.47 g</td>
</tr>
<tr>
<td>Magnesium Chloride (anhydrous)</td>
<td>10.93 g</td>
</tr>
<tr>
<td>Malachite Green Oxalate</td>
<td>37.0 mg</td>
</tr>
<tr>
<td>Agar</td>
<td>2.7 g</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.

**Difco™ Novobiocin Antimicrobial Supplement**

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula per 10 mL Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Novobiocin</td>
<td>20.0 mg</td>
</tr>
</tbody>
</table>

Directions for Preparation from Dehydrated Product

**Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification**

1. Suspend 31.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE. Cool to 50°C.
3. Aseptically add 10 mL of Novobiocin Antimicrobial Supplement. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

**Difco™ Novobiocin Antimicrobial Supplement**

Rehydrate with 10 mL sterile purified water. Mix well.
User Quality Control

Identity Specifications
Difco™ Rappaport-Vassiliadis Medium, Semisolid Modification

Dehydrated Appearance: Pale green, free-flowing, homogeneous.
Solution: 3.16% solution, soluble in purified water upon boiling. Solution is blue, clear to slightly opalescent.
Prepared Appearance: Blue, slightly opalescent, semisolid.
Reaction of 3.16% Solution at 25°C: pH 5.2 ± 0.2

Cultural Response
Difco™ Rappaport-Vassiliadis Medium, Semisolid Modification

Prepare the medium per label directions. Inoculate and incubate at 42 ± 0.5°C for 18-24 hours.

To confirm a presumptive identification of Salmonella:
Rapid serologic confirmation
1. Inoculate M Broth with growth from migration edge on MSRV, Semisolid Modification plate.
2. Incubate at 35°C for 4-6 hours (until turbid). M Broth culture can be held for up to 24 hours at 35°C.
3. Test with Salmonella O and H antisera.

Culture confirmation
1. Transfer a loopful of growth from the migration edge on MSRV, Semisolid Modification plate onto Hektoen Enteric Agar and streak for isolation.
2. Incubate at 35°C for 24 ± 2 hours.
3. From colonies of Hektoen agar that show colony appearance typical of Salmonella (green colonies with black centers), perform biochemical tests to confirm the identification.

Limitation of the Procedure
The combination of malachite green, magnesium chloride and a low pH may inhibit certain Salmonella, such as Salmonella Typhi and Salmonella Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

Procedure
Pre-enrichment
1. Add 25 g of cocoa or chocolate to 225 mL of sterile reconstituted nonfat dry milk with 0.45 mL of a 1% aqueous brilliant green dye solution; mix well.
2. Incubate at 35°C for 20 ± 2 hours.

Selective Enrichment
3. Inoculate 10 mL Tetrathionate Broth (prewarmed to 35°C) with 1 mL of the pre-enrichment culture.
4. Incubate at 35°C for 8 ± 0.5 hours.

Motility Enrichment on MSRV, Semisolid Modification
5. After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSRV plate.
6. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Expected Results
Positive: Growth of migrated cells is visible as a gray-white, turbid zone extending out from the inoculated drop. Test sample is considered presumptively positive for motile Salmonella.

Negative: Medium remains blue-green around the drops, with no gray-white, turbid zone extending out from the drop. Test sample is considered negative for motile Salmonella.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>HALO/ MOTILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrobacter freundii</td>
<td>8090</td>
<td>10^2-10^3</td>
<td>Marked</td>
<td>–</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>9240</td>
<td>10^2-10^3</td>
<td>None</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>27853</td>
<td>10^2-10^3</td>
<td>None</td>
<td>–</td>
</tr>
<tr>
<td>Salmonella enterica subsp. enterica serotype Enteritidis</td>
<td>13076</td>
<td>10^2-10^3</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella enterica subsp. enterica serotype Typhimurium</td>
<td>14028</td>
<td>10^2-10^3</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella senftenberg (NCTC 10384)</td>
<td>10^2-10^3</td>
<td>Good</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Difco™ & BBL™ Manual, 2nd Edition
Availability
Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification
CCM
Cat. No. 218681  Dehydrated – 500 g

Difco™ Novobiocin Antimicrobial Supplement
AOAC
Cat. No. 231971  Vial – 6 x 10 mL*
*Store at 2-8°C.